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Bartkova, Simona; Kokotovic, Branko; Dalsgaard, Inger

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DETECTION OF *AEROMONAS SALMONICIDA* IN FISH TISSUE BY REAL-TIME PCR

S. Bartkova*, B. Kokotovic, I. Dalsgaard
National Veterinary Institute, Technical University of Denmark, Frederiksberg C, Denmark

Background

In Denmark rainbow trout (*Oncorhynchus mykiss*) are transferred from freshwater out to the sea, where outbreaks of furunculosis caused by the bacterium *Aeromonas salmonicida* subsp. *salmonicida* occur during elevated water temperatures [1]. One therefore speculates that some fish "carriers" might harbor the bacterium as a latent infection from freshwater to the sea [2]. Since past research involving carrier fish includes slow and laborious enrichment steps [3,4] a sensitive and simple method for detection of the carriers is highly needed.

Objective

To develop a highly sensitive, rapid and cost-effective real-time PCR assay that detects *A. salmonicida* in various tissue samples from not only rainbow trout showing clinical symptoms, but possible "carriers" as well.

Results

A highly sensitive and specific real-time PCR has been developed, based on previous research by Balcázar et al. [5]. The assay uses a self-quenched fluorogenic primer set designed from a DNA probe sequence for *A. salmonicida*, which is the most frequently used target for species-specific *A. salmonicida* molecular methods to date [5].

Specificity

Balcázar et al. [5] amplified DNA from all 16 *A. salmonicida* isolates tested, while all 26 non-*A. salmonicida* produced no product. In this study we also amplified DNA from all 28 Danish *A. salmonicida* isolates tested.

Sensitivity

When tested on five different rainbow trout tissue samples (gills, kidney, brain, intestine, spleen) spiked with various *A. salmonicida* dilutions, the assay showed no sign of inhibition.

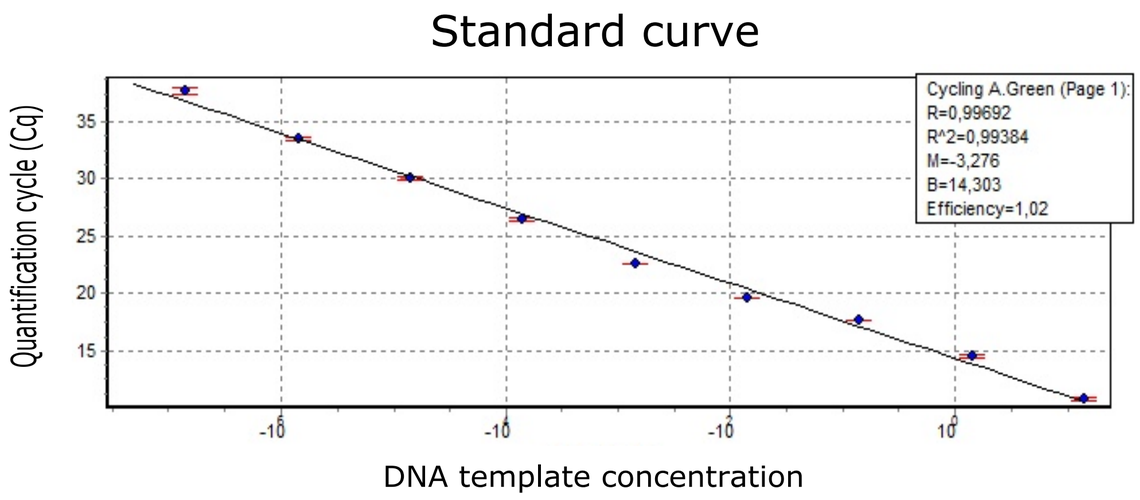


Fig 1: Standard curve plot constructed with serial 10-fold dilutions of 14.3 ng/µL to 1.43x10-7 ng/µL of the purified *A. salmonicida* type strain ATCC 33658 recombinant plasmid DNA.

Table 1: Bacteriology and real-time PCR assay results* from rainbow trout field samples at a Danish sea farm. The fish had an outbreak of furunculosis and were treated with antibiotics. The treatment finished 5 days before sampling.

Fish #	Organs					Notes
	Gills	Spleen	Intestine	Kidney	Brain	
09	- / +	- / +	- / +	- / +	- / +	*A .salmonicida* was isolated from the eye with bacteriology
13	+ / +	- / +	- / -	- / +	- / +	-
17	+ / +	- / +	- / ±	- / +	- / ±	-
23	+ / +	+ / +	+ / +	+ / +	+ / +	-
29	+ / +	+ / +	- / ±	+ / +	- / +	Dead
30	- / +	- / +	- / +	- / ±	+ / +	Moribund
31	+ / +	- / +	- / +	- / +	- / +	-
32	+ / +	+ / +	- / +	+ / +	+ / +	-
39	+ / +	- / +	- / +	- / +	- / +	-
42	+ / +	+ / +	- / +	+ / +	- / +	-
15	- / +	- / +	- / +	- / +	- / ±	Moribund
22	- / ±	- / ±	- / ±	- / -	- / +	Moribund
25	- / -	- / +	- / ±	- / +	- / ±	Moribund
26	- / ±	- / +	- / ±	- / +	- / -	Moribund
28	- / -	- / +	- / -	- / +	- / +	Moribund
37	- / -	- / +	- / +	- / +	- / +	Had "pop-eye"

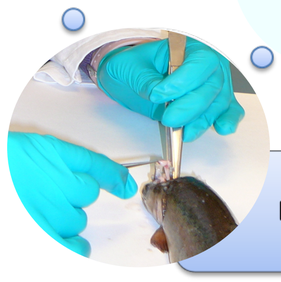
* **Bacteriology result / qPCR result**
- Negative result
+ Positive result
± Uncertain qPCR result (i.e. either the average Cq of the sample replicates was higher than 38 or not all of the sample replicates produces a signal above the Cq threshold)

Methods

Bacteriology
Time: Minimum 48 hours incubation time

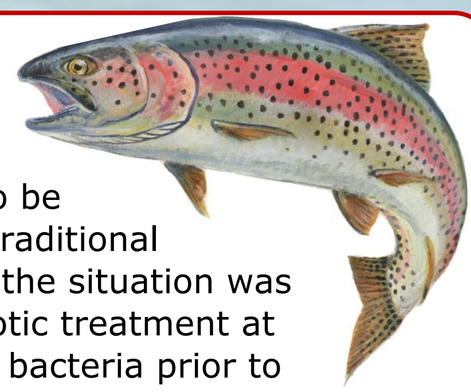
Run a real-time PCR using Rotor-Gene Q
Time: 1.45 hours (20 samples per run in triplicates)

DNA extraction using Instagene matrix
Time: 1.30 - 2 hours in total (10-12 samples)



Extraction of five organs

Conclusion



Preliminary results from a natural occurring outbreak show that the assay seems to be more sensitive compared to traditional bacterial methods. However, the situation was not optimal due to the antibiotic treatment at the fish farm that killed most bacteria prior to sampling.The dead bacteria could thus not be cultured with bacteriology while its DNA could be amplified by the real-time PCR. This is especially noticeable in the moribund fish # 15-37 where all bacteriology results are negative, while most of the real-time PCR results are positive. More testing thus needs to be done before drawing any firm conclusions. Nevertheless, if further results do show the same pattern as illustrated in fish # 09-42, this real-time PCR assay could become a vital part in detection of *A. salmonicida*. Particularly for finding carriers or latent infections.

References

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